



Customer Case Study: Seed Biotechnology Center, University of California, Davis

Pepper – *Capsicum annuum*

Capsicum spp. is an important vegetable and spice crop worldwide and shows incredible diversity in its many varieties.

As with most commercial crops, pepper is the subject of many breeding programs. These programs aim to enhance valuable traits in the crop such as improved productivity and resistance to biotic and environmental stresses, as well as consumer characteristics such as taste and colour. For pepper breeders, many of the traits of interest in current *Capsicum* breeding programs include complex, multi-genic traits that are not easily integrated through traditional breeding strategies.



Phytophthora capsici – a problem for all seasons

One of the biggest problems the pepper industry faces is *Phytophthora capsici* (*Pc*), root rot or late blight, from which most commercial pepper varieties suffer yield losses despite good management practices and resistance strategies.

The lifecycle of *Phytophthora* makes it particularly difficult to get rid of once it infects, and many farmers are forced to abandon their crops if it appears. Selective breeding of varieties which naturally carry *Phytophthora* resistance is an attractive long-term solution but is complicated by the dynamic array of races of *Phytophthora* found in fields over time, and variable resistance across plant varieties and tissue types.

Identifying and selectively breeding for pepper strains resistant to *Pc* is exactly what Allen Van Deynze and his fellow researchers at the Seed Biotechnology Center have set about achieving with significant progress, as reported in their recent paper “*CaDMR1* cosegregates with QTL *Pc5.1* for resistance to *Phytophthora capsici* in Pepper (*Capsicum annuum*)”¹.

The group at the Seed Biotechnology Center are applying the most up to date tools and understanding of genetics, genomics and molecular biology to characterise peppers and their diverse characteristics. This, in turn, enables introgression of valuable complex traits, such as disease resistance, into the available breeding stock while keeping other characteristics, such as shape, size and taste, that are familiar to the consumers.

The goal of the research reported in Rehrig *et al.*¹ was to identify genes associated with resistance to root rot in pepper. Their approach was to screen a pepper population that exhibited *Pc* resistance and to track common regions in the genome (Quantitative Trait Loci or QTL), and then the genes themselves, that confer the resistance using SNPs (single nucleotide polymorphisms) as genetic markers.

The study identified a gene that encodes an enzyme which is highly associated with *Pc* resistance in pepper; this is the first report to relate candidate genes to a known *Pc* resistance factor in pepper.

High-throughput polymorphism detection and gene expression analysis – the first step

Major progress in pepper breeding was announced by the team in 2013, when the Seed Biotechnology Centre and the Genome Centre published a paper describing a method for high-throughput polymorphism detection and application in the pepper². Taking advantage of recent *Capsicum* EST sequencing efforts³ along with a custom genotyping array design, hybridisation methods and algorithms⁴, an Affymetrix Pepper GeneChip[®] was designed based on > 30,000 pepper genes and the array was then used to identify and analyse polymorphisms across 43 different *Capsicum* lines and *Capsicum* species.

Tracking down genetic determinants of *Pc* resistance

Then, as reported in the study, a high density map with 3892 SNP markers was generated in a set of recombinant inbred lines derived from the highly *Pc* resistant strains (*Capsicum annuum* accession Criollo de Morelos-334 (CM334) and Early Jalapeño). The lines were intensively screened for root rot resistance

against *Pc* isolates from across North and South America. QTL effective across, and specific to, isolates were identified and SNP markers which were associated with these resistance QTL were designed and validated across different *Pc*-resistant populations.

Using the genomic sequence of pepper, they examined genes found within the genetic region conferring resistance and found a gene that encodes an enzyme that is highly associated with resistance in pepper.

Now validated as markers of *Pc* resistance, the identified SNPs can be used for targeted breeding and selection of resistant commercial pepper strains with huge potential benefits for both breeder and consumer in the future.

How KASP SNP genotyping was used

In the final stages of this research, QTL and finally a sub-set of SNP markers were identified which acted as genetic markers for *Pc* resistance in multiple pepper strains.

At the point where the team had narrowed their selection of SNP markers down, the array chip was no longer practical and the group switched to using KASP genotyping to follow up on the candidates and to perform the crucial functional validation of them as *Pc* resistance markers.

The research team at the Seed Biotechnology Center used LGC to perform KASP genotyping of 30 gene markers in 250 pepper lines, as well as an additional 10 markers in 250 lines out of our US service labs in Beverly, MA.

The benefit of using KASP SNP markers

The group used LGC's KASP genotyping service to generate the SNP genotype data they required for the functional validation of selected SNP markers.

"LGC's high-throughput and fast turnaround times (40 SNP markers each run on 250 different plant lines in 6 weeks) produced high-resolution data that was crucial for validating our research and getting it published ahead of the field." said Van Deynze.





Seed Biotechnology Center at UC Davis

Allen Van Deynze is director of research at the Seed Biotechnology Center, UC Davis - a leading light for modern plant breeding across both the academic and commercial plant breeding communities. UC Davis is the number one school in the world for agriculture and food studies and is the most cited for scientific publications (QS World University Rankings).

The Seed Biotechnology Center's research team deciphers the genomes of many common crops in order to link plant genes to desirable characteristics which can lead to improved varieties. Crops such as carrots, cotton, lettuce, melons, peppers, and spinach are investigated and improved, often in partnership with key breeders of these crops.



References

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4. Stoffel, K., van Leeuwen, H., Kozik, A., Caldwell, D., Ashrafi, H., Cui, X., ... & Van Deynze, A. (2012). Development and application of a 6.5 million feature Affymetrix Genechip® for massively parallel discovery of single position polymorphisms in lettuce (*Lactuca spp.*). *BMC genomics*, 13(1), 185.



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With headquarters in Teddington, South West London, LGC employs over 2,000 staff, operating out of 22 countries worldwide. Its operations are extensively accredited to international quality standards such as ISO/ IEC 17025.

Set up in 1842 as the Laboratory of the Government Chemist, for more than 100 years LGC has held the unique function of the Government Chemist in the UK. LGC was privatised in 1996 and is now majority-owned by funds managed by Bridgepoint.

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